# Predator Odors as Repellents to Brushtail Possums and Rabbits

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## **ABSTRACT**

Repellents are being developed as an alternative to the use of poisons, traps, or firearms for controlling damage to forest and farm plantings by the introduced Australian brushtail possum (Trichosurus vulpecula) and European rabbit (Oryctolagus cuniculus). Such repellents need to protect seedlings from irreversible damage for at least 6 months after application. Seven synthetic predator odor compounds were compared with "Treepel," a moderately effective commercial repellent, by assessing relative browse on treated Pinus radiata seedlings in pen tests. Predator odors were repellent to both animals. Generally they were more repellent to possums than to rabbits, but the predator odor-based TOM (formulation confidential) was particularly repellent to rabbits. In a longer field trial at a site heavily infested with rabbits, both TOM and Treepel gave good initial protection, TOM being more effective. However, effectiveness declined after 56 days, and extensive browning was affecting foliage, particularly after treatment with TOM. The formulation of TOM therefore needs refinement to prevent phytotoxicity and to prolong effectiveness. The results support the existence of a sensory mechanism that enables herbivores to avoid predators by detecting by-products of meat-eating animals. If this mechanism is innate for all herbivores rather than interspecific for particular herbivores and predators, development of broad-spectrum herbivore repellents may be possible.

# **KEYWORDS**

repellents, damage by wildlife, vertebrate pests, possums, rabbits, New Zealand, browsing

# INTRODUCTION

Each year, 50–100,000 ha of commercial forest are planted in New Zealand, with over 90% comprising *Pinus radiata*. In many areas, introduced herbivore pests have a significant impact on the survival of newly planted *P. radiata* seedlings, so that pest control operations are required before and sometimes after planting. The main pests responsible are the Australian brushtail possum (*Trichosurus vulpecula*), the European rabbit (*Oryctolagus cuniculus*), and, to a lesser extent, the brown hare (*Lepus europaeus occidentalis*). Typically, possums and rabbits browse

seedlings until the plant is eaten to ground level, but hares often remove only the apical tip. Although damaged plants can recover, they are usually worthless because of resultant poor growth and form.

Chemical repellents are used widely to reduce pest damage to plants, either in combination with, or as an alternative to, pest control. Compared with pest control techniques such as poisoning and shooting, repellents are usually safer to humans and nontarget animals and, therefore, are also suited to other applications such as protection of farm shelter belts, orchards, or domestic gardens. In New Zealand, only two repellent products are available, of which Treepel (Aorangi Forestry Services Limited, Waimate) is the most widely used. This egg-based repellent, formulated in an acrylic liquid adhesive, can be effective against rabbits and hares for up to 2 months (Crozier 1991). However, we surveyed the requirements of foresters, farmers, local authorities, and plant nursery managers and found a need for safe repellents that will give protection after just one application for 6 months after planting, at a cost of no more than U.S. 10 cents per seedling.

Predator odors based on urines, feces, or synthetic components of naturally occurring secretions are often repellent to herbivores (for reviews see Weldon 1989 and Nolte et al. 1994), and sulphurous by-products of meat digestion may be the cues by which herbivores detect and avoid predators (Nolte et al. 1994). In this paper we compare the effectiveness of seven predator odors in reducing possum and rabbit damage to *Pinus radiata* seedlings tested against Treepel, on the basis that new repellents would need to be at least as effective as existing ones. One of these compounds was also tested in the field.

#### **METHODS**

## **Materials**

Six sulphur-containing compounds previously identified from animal secretions (Vernet–Maury et al. 1984) were synthesized. The component from fox (*Vulpes*) feces most repellent to rats, 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), was prepared using the methods described by Sullivan et al. (1988). Components of the anal gland secretions of the genus *Mustela*, iso-pentanylmethylsulfide (PMS), 2-propylthietane (PPT), 3-propyl-1,2-dithiolane (PDT), 2,2-dimethylthietane (DMT), and 3,3-dimethyl-1,2-dithiolane (DMD), were prepared using the methods given by Crump (1978, 1980, 1982). Except for PMS, these compounds are also found in fox feces (Vernet–Maury et al. 1984). We synthesized 20–50 g of these six compounds and formulated them for pen-testing as 5% m/v solutions in medicinal grade paraffin oil suitable for spray application. This formulation has been shown to be an effective concentration of one of the compounds (DMT) for repelling rabbits and other small mammals (Robinson 1990). We also tested a predator formulation TOM that may include sulphur. Details of this formulation are presently unavailable for commercial reasons. All seven predator odors were tested against possums and rabbits.

## Pen Trials

Three adjacent animal pens (each 30 m x 5 m), with open gates between them to permit free access, were used in all trials (Figure 1). In two series of trials, six possums and 15-20 rabbits were used to test the compounds. Pens had a sheltered area with nest boxes at one end where possums rested during the daytime. Rabbits rested in ground cover and metal shelters. Supplementary feed pellets and apples, as well as the grass and clover growing in the pens, and a supply of fresh water were always available.

In each trial a row of seedlings was planted in the two outer pens. Predator odors were applied to trees in one of these (randomly selected) and Treepel as a positive control treatment in the other (see below). No seedlings were planted in the center pen so that treated and control groups were separated. This arrangement was designed to simulate field application of repellents to groups of seedlings, where each treated seedling reinforces the repellency of the group. The number of seedlings planted was varied to ensure similar feeding pressure on seedlings throughout the trials. When trials started in winter, 25 seedlings approximately 25-cm high were planted in each row; but in summer trials, only 8 that were planted as seedlings were approximately 60 cm high by this stage. This seedling density allowed a feeding pressure that was deliberately greater than expected in the wild to ensure speedy results.

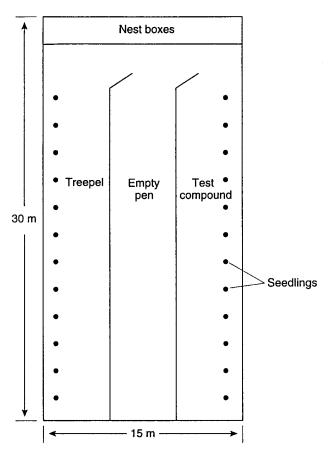


FIGURE 1. Layout of pen trials of repellents showing the planting design of seedlings and application of treatments.

In the first and last possum and rabbit trials, untreated seedlings were planted in both end pens to test the assumption that the animals did not prefer feeding in either pen. In the second trial with each animal species, the repellent action of Treepel was evaluated and confirmed (Tables 1 and 2). Consequently, the results from this trial were then used as a positive control in the other trials testing predator odors. A minimum of 3 days was allowed between trials to reduce the possibility of possums and rabbits becoming habituated to predator odors.

The height of each seedling was measured directly after planting; then the percentage of each seedling left unbrowsed was estimated daily until seedling foliage was reduced to about 10% in one of the pens. This avoided the confounding effect of treatments being subject to additional browsing pressure when the more acceptable alternatives had been browsed out. The duration of each trial was 5–10 days. Trials were started during fine weather so that repellents adhered to dry foliage.

The percentage of each seedling left unbrowsed each day was plotted for each day of the trial. The mean area under the curve (AUC) for treatments was divided by the mean AUC for controls as a combined measure of treatment effectiveness and durability in relation to Treepel. Values of more than 1 for this parameter, the "mean AUC ratio," indicated that treatments were more effective than the positive control. The mean AUC's for treatments and controls were also compared by t-test to determine the significance of any differences in effectiveness.

An index of effectiveness (I) was calculated by dividing the mean AUC ratio for predator odors: Treepel by the mean AUC ratio for untreated trees: Treepel, and testing for difference between the two ratios by t-test. This could have been assessed more directly by presenting untreated trees in each trial instead of Treepel-treated trees, but our main objective was to assess effectiveness relative to an established repellent product. Simultaneous inclusion of a negative control may have confounded the comparisons with Treepel.

# **Field Trial**

Based on the results of the pen trials, a field trial was conducted during spring-summer 1994 to determine the effectiveness of TOM and Treepel as rabbit repellents. A site was selected at Eyrewell Forest, North Canterbury, a plantation forest supporting locally high numbers of rabbits. Plantings during the winters of 1992 and 1993 had both been unsuccessful because of extensive rabbit browsing, despite the implementation of control programs. A site measuring  $1,000 \times 140$  m was selected, bounded by mature *P. radiata* forest. The sandy shingle ground was sparsely vegetated with grasses, weeds, and low shrubs. Three treatments (TOM, Treepel, and no treatment) were applied to groups of 200 *P. radiata* seedlings, each planted 2 m apart on 10 rows 5 m apart (Figure 2). Seedlings measured 25–40 cm above ground height after planting. Treatments were replicated four times throughout the site. Treepel or TOM was applied to the seedlings by immersing the foliage of groups of 30–40 seedlings at a time in a bucket of the compound within 1 hr before planting. This treatment method was recommended by the manufacturers of both compounds as a more efficient alternative to spray treatment.

Damage to seedlings was assessed after 4, 11, 22, 55, and 81 days. Seedlings were classified as being significantly damaged if growing tips and more than 50% of the foliage were removed or the plant had been pulled out of the ground. Less severe damage has no effect on tree

Table 1. Tests for Pen Preference (No. of Treatments) at Start and End of Trials, and Effectiveness of Treepel as a Positive Control. A Mean

| AUC Ratio of<br>Differences       | 1 Indicates Eq       | uality of Treatmo | ents. Differ   | ences Were Teste | AUC Ratio of 1 Indicates Equality of Treatments. Differences Were Tested by t-Test and the $ ho$ Va Differences | e P Value Indicat | alue Indicates Significance of the | ce of the |
|-----------------------------------|----------------------|-------------------|----------------|------------------|---|-------------------|------------------------------------|-----------|
|                                   |                      | Possur            | Possum trials  |                  |   |                   | Rabbit trials                      |           |
| Treatments                        | No. Trees<br>per Pen | Mean AUC<br>Ratio | <b>~</b>       | P                | No. Trees<br>per Pen  | Mean AUC<br>Ratio | <b>.</b>                           | ٩         |
| Untreated in both pens (at start) | 25                   | 1.08              | <u>.1</u><br>ω | 0.21             | 1<br>5  | 0.96              | 0.9                                | 0.39      |
| Treepel v. untreated              | 25                   | 1.45              | 7.2            | < 0.001          | ∞   | 3.08              | 10.9                               | < 0.001   |
| Untreated in both                 | 15                   | 1.04              | 1.2            | 0.34             | 10  | 1.11              | 1.2                                | 0.32      |

pens (at end)

TMT

25

0.60

< 0.001

œ

-10.8

< 0.001

Table 2. Effectiveness of Predator Odors Relative to Treepel in Pen Trials With Possums and Rabbits. The Mean AUC Ratio Indicates

Fifectiveness if a Values Above 1 Indicate Greater Effectiveness Than Treepel) and the P Value Indicates Significance of the

| Differences Co                            | mpared M | is Above 1 Indicate G<br>Vith Treepel by t-Test  Possum trials | ate Greater Eff<br>Test. Treatmo | Effectiveness (i.e., Values Above 1 Indicate Greater Effectiveness Than Treepel) and the P Value Indicates Significance of the Differences Compared With Treepel by t-Test. Treatments are Listed in Order of Repellency Towards Possums  Rabbit trials | reepel) and the<br>Order of Repelle | P Value Indicates ncy Towards Pos R | es Significance of Ssums Rabbit trials | ine     |
|---|----------|--|----------------------------------|---|-------------------------------------|-------------------------------------|--|---------|
| Predator Odor Tested<br>(Control=Treepel) | n        | Mean AUC<br>Ratio  | <b>~</b>                         | P   | 3                                   | Mean AUC<br>Ratio                   | <b>~</b>                               | ρ       |
| DMD                                       | 15       | 2.29   | 12.2                             | < 0.001   | œ                                   | 0.83                                | -6.9                                   | < 0.001 |
| PPT                                       | 25       | 1.78   | 9.1                              | < 0.001   | œ                                   | 0.83                                | -6.0                                   | < 0.001 |
| PMS                                       | 25       | 1.30   | 2.86                             | 0.006   | 10                                  | 0.86                                | -1.1                                   | 0.302   |
| DMT                                       | 25       | 1.13   | 1.45                             | 0.15  | œ                                   | 0.54                                | -16.4                                  | < 0.001 |
| ТОМ                                       | 10       | 1.11   | 3.38                             | < 0.001   | 10                                  | 3.70                                | 12.5                                   | < 0.001 |
| PDT                                       | 25       | 1.03   | 0.45                             | 0.65  | 10                                  | 0.86                                | -4.3                                   | 0.002   |

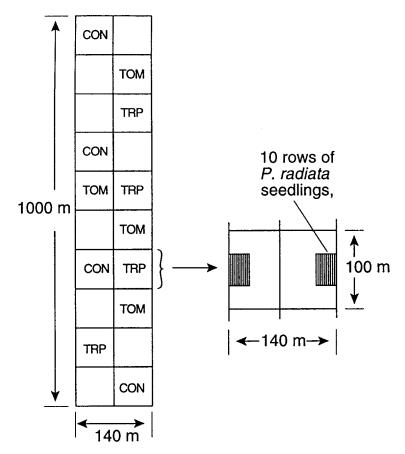


FIGURE 2. Layout of field trial of two repellents showing the distribution and replication of treatments, and planting design of seedlings in each of the treatment blocks.

growth 3 years after planting (Neilson 1981). It is essential that repellents do not damage seedling foliage, so the degree of browning of the foliage was recorded where it exceeded more than 25%, as a conservative estimate of the minimum amount of browning that may affect seedling growth.

Browse and foliage-browning on the three treatments over time were compared by repeat measures ANOVA.

# **RESULTS**

## **Pen Tests**

Possums and rabbits showed no preference for either pen used for presenting seedlings, both at the beginning and the end of the trials, and Treepel was confirmed as being repellent to both species, particularly rabbits (Table 1).

Most of the predator odors were highly repellent to possums (Table 2). Four of the compounds were significantly more repellent than Treepel, the most effective being DMD, which still retained its activity at the end of the trial when the control seedlings had been almost completely eaten. TOM, PPT and IPMS were also shown to be more repellent to possums than the control, but seedlings treated with PDT and DMT were browsed to the same extent as the Treepel-treated plants. TMT was significantly less repellent than Treepel.

In tests with rabbits (Table 2), only TOM proved significantly more effective than Treepel, retaining its repellency when the control seedlings had been almost completely eaten. The other predator odors were less effective against rabbits than they were against possums. Nevertheless, for both species all but one of the I values, indicating effectiveness relative to no treatment, was significant (Table 3). Therefore all predator odors, except TMT tested against possums, gave some degree of protection against possum and rabbit browsing.

Although the animals were exposed to up to seven predator odors, there was no evidence of either habituation towards, or a reinforcing effect of, successive treatments. For example, possums responded most aversively towards the final treatment (DMD) but least aversively towards the penultimate treatment (PDT). Similarly, the rabbits' strong aversive response towards TOM was preceded and followed by a lack of repellency in DMT and PDT, respectively.

# **Field Trial**

Repellent-treated trees (treatments combined) suffered significantly less damage than untreated trees over the duration of the trial (t=8.6, d.f.=9, P<0.001; Figure 3). More than one-third of the untreated trees were heavily browsed or pulled out of the ground after 10 days, but few repellent-treated trees were damaged. However, after 81 days, when only about 10% untreated trees were not heavily browsed, 25–35% of repellent-treated trees were also heavily damaged or pulled out of the ground. The difference in damage between trees treated with TOM and Treepel was not statistically significant (t=0.75, d.f.=6, P=0.47).

Table 3. Index of Effectiveness (I) of Predator Odors Relative to No Treatment in Pen Trials With Possums and Rabbits. The Index Was Calculated by Comparing the Mean AUC Ratios for Predator Odors:Treepel and No Treatment:Treepel, and Significance Determined by t-Test

| Predator Odor |     | Possum T | rials   |      | Rabbit Tria | ls      |
|---------------|-----|----------|---------|------|-------------|---------|
| Tested        | ı   | t        | P       | 1    | t           | P       |
| DMD           | 3.2 | 10.1     | < 0.001 | 2.5  | 7.5         | < 0.001 |
| PPT           | 2.6 | 2.1      | 0.05    | 2.5  | 7.5         | < 0.001 |
| PMS           | 1.9 | 4.4      | < 0.001 | 2.6  | 3.5         | 0.005   |
| DMT           | 1.6 | 3.7      | < 0.001 | 1.6  | 3.2         | 0.008   |
| TOM           | 1.6 | 3.4      | 0.002   | 11.2 | 6.8         | < 0.001 |
| PDT           | 1.5 | 2.5      | 0.015   | 2.6  | 8.0         | < 0.001 |
| TMT           | 0.9 | -1.2     | 0.25    | 1.6  | 3.4         | 0.005   |

Although seedlings initially showed little sign of phytotoxicity, foliage on most trees treated with TOM showed extensive browning or the trees were dead after 12 weeks, and about one-fourth of Treepel-treated trees were similarly affected (Figure 4). Foliage browning and resultant death affected significantly more TOM-treated trees than Treepel-treated trees. The difference was first evident after 56 days (t = 2.8, d.f. = 6, P = 0.04) and became more conspicuous by 81 days (t = 4.6, d.f. = 6, P = 0.005). Insufficient untreated trees remained after 10 days for us to assess whether foliage browning was due solely to repellent treatment.

# DISCUSSION

All predator odors protected seedlings against browsing by possums and rabbits to some extent, except TMT against possums, indicating that the predator odors tested are potentially suitable for further development as repellents for possums and rabbits. In short-term pen trials, predator odors repelled both possums and rabbits, although they were more effective against possums than rabbits. The most effective compound against possums in the pen trials was DMD, but TOM proved superior to Treepel against both species and was selected for the field trial.

The field trial was a demanding test of repellents, as indicated by the almost complete and rapid destruction of untreated trees by rabbits. The superiority of TOM over Treepel found in pen trials with rabbits was not confirmed in the field trial, and it did not persist for the 6 months required. Nevertheless, these initial results indicate that, with some refinement, TOM should achieve superior performance to Treepel in the field. Studies are underway on the repellency and phytotoxicity of TOM over time at different concentrations and in slower release formulations.

The "dipping" method of applying compounds recommended by manufacturers may have been responsible for some of the foliage browning recorded. Since untreated trees did not survive long enough to permit assessment of the contribution of other factors, particularly the dry conditions experienced in the second half of the trial, we cannot ascertain whether all of this recorded damage can be attributed to phytotoxic effects of the treatments. However, the degree of foliage browning differed significantly for TOM- and Treepel-treated seedlings, suggesting that the repellent treatment was at least partly responsible and that, of the two treatments, TOM was the more harmful. Trees saturated in a complete coating of repellent may face more difficulty in maintaining normal physiological functions than trees with a lighter spray delivered coating, especially in times of stress. This needs further assessment. In such field trials it would be advisable to physically protect untreated trees from herbivore browsing so that the phytotoxicity of repellents can be assessed directly.

The effectiveness of predator odors may result from conditioned responses of herbivores to specific predators (Swihart et al. 1991), innate generalized responses to common sulphurous byproducts of meat-eating (Nolte et al. 1994), or perhaps from a combination of both. Although possums in New Zealand could have learned to avoid mustelids by odor detection, as they have been sympatric with them for some 130 years, such a response to foxes could not have been learned. If predator odor avoidance is a conditioned response, this might explain why TMT (fox odor) was the least effective repellent in the possum pen trials. Rabbits, however, evolved



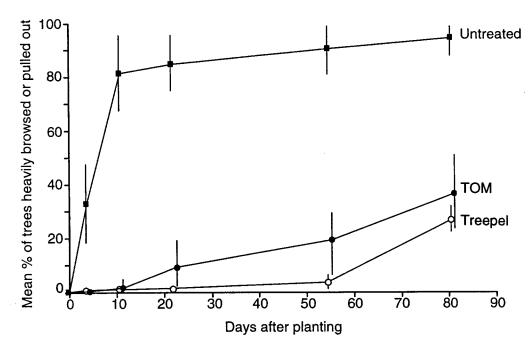


FIGURE 3. The mean percentage of trees ( $\pm$ SE) heavily browsed or pulled during the 81 days of the field trial comparing TOM, Treepel, and no treatment.

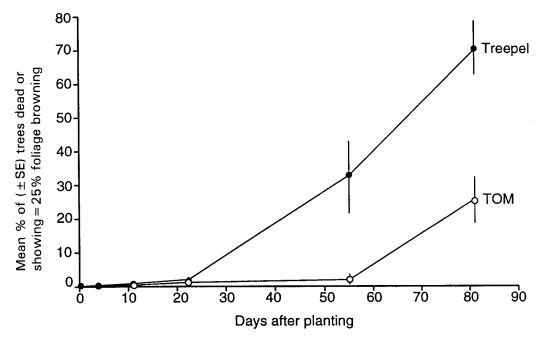


FIGURE 4. The mean % of percentage of trees ( $\pm$  SE) with heavy browning of the foliage during the 81 days of the field trial comparing TOM, Treepel, and no treatment (CON).

sympatrically with mustelids and foxes, and we are unable to explain why they showed a weaker aversive response than possums to most of the predator odors. To further examine the alternative "innate-generalised response" hypothesis, we intend to test the urine of Australian marsupial predators against possums (with which they are sympatric) and rabbits (allopatric). A positive result against both species would tend to support the hypothesis and reinforce the concept of development of broad-spectrum herbivore repellents based on predator odors.

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